ABSTRACT

Candidate: Bc. Jana Kolářová

Supervisor: Mgr. Klára Konečná

Supervisor – specialist: PharmDr. Ivona Pávková, Ph.D

Title: Isolation and proteome analysis of fractions enriched in periplasmic proteins of bacterium *Francisella tularensis*

Diploma thesis

Charles University in Prague, Faculty of Pharmacy in Hradec Králové,

Department of Biological and Medical Sciences

Study field: Special worker in laboratory methods

The aim of the presented diploma thesis was to test several previously published methods for preparation of fractions enriched in periplasmic proteins of bacterium *Francisella tularensis*. The selected method will be further used in studies aimed at finding potential substrates of hypothetical protein with homology to DsbA as several of them are expected to play a crucial role in *Francisella tularensis* virulence. DsbA proteins found in periplasm of gramnegative bacteria are in general responsible for disulfide bond introduction and thus for right folding and function of number of extracytosolic proteins, known virulence factors included.

Tested methods included the method of cold osmotic shock, the method using chlorofom, polymyxin B sulphate, EDTA in combination with lysozyme and magnesium chloride in combination with lysozyme. The obtained protein samples were separated using two-dimensional gel electrophoresis and the acquired 2-D protein maps were analyzed using the ImageMaster 2D Platinum software. The suitability of method was evaluated first by the enrichment in proteins with predicted periplasmic localization and by the differences in protein synthesis between the wild type strain and mutant strain with deletion of gene encoding the lipoprotein homological to DsbA protein.

The method of cold osmotic shock and method using chloroform seem to be the most suitable methods. However, the suitability and especially the reproducibility of these methods have to be further tested and confirmed.